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Title: Long-term follow-up in adrenal incidentalomas: an Italian Multicenter Study

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Abstract

Context. The long-term consequences of subclinical hypercortisolism (SH) in patients with adrenal incidentalomas (AI) are unknown.

Setting-Patients. In this retrospective multicentric study, 206 AI patients with a ≥5 yrs follow-up (median, range: 72.3, 60-186 months) were enrolled.

Intervention-Main Outcome Measure. The adrenocortical function, adenoma size, metabolic changes and incident cardiovascular events (CVE) were assessed. We diagnosed SH in 11.6% of patients, in the presence of cortisol after 1mg-dexamethasone suppression test (1mg-DST) >5 µg/dL (138 nmol/L) or at least 2 out of: low ACTH, increased urinary free cortisol and 1mg-DST >3 µg/dL (83 nmol/L).

Results. At baseline, age, CVE and type-2 diabetes (T2DM) prevalence were higher in patients with than in patients without SH (62.2±11yrs *vs* 58.5±10yrs; 20.5% *vs* 6%; 33.3% *vs* 16.8%, respectively, P<0.05). SH and T2DM were associated with prevalent CVE (OR 3.1, 95%CI 1.1-9.0 and OR 2.0, 95%CI 1.2-3.3, respectively) regardless of age. At the end of the follow-up, SH was diagnosed in 15 patients without SH at baseline. Weight, glycemic, lipidic and blood pressure control worsened in 26%, 25%, 13% and 34% of patients, respectively. A new CVE occurred in 22 patients. SH was associated with the worsening of ≥2 metabolic parameters (OR 3.32, 95%CI 1.6-6.9) and with incident CVE (OR 2.7, 95%CI 1.0-7.1) regardless of age and follow-up.

Conclusion. In AI patients a clinical-biochemical long-term FU is recommended for the risk of SH development. In SH patients the risk of worsening of the metabolic control and CVE has to be considered in addressing the best approach.

Introduction

In the last decades, the wide use of radiological techniques has brought to the detection of asymptomatic incidental adrenal masses, named “adrenal incidentaloma” (AI) in 1% to 4.2% of the general population (1-2). At the diagnosis the majority of these masses are benign and non-functioning adenomas. However, the natural history of these tumors is only partially known (3-5).

Available data suggest that in 5% to 20% of patients a >1 cm adrenal mass enlargement occurs after a mean follow-up period of 4 years, regardless of adrenal function. Nevertheless, the risk to develop malignancy in AI patients is very low (<1/1000) (6-10). Furthermore, in 5% to 30% of AI patients, depending on the different criteria used to define SH, a condition of subclinical hypercortisolism (SH) is present, which is defined by the presence of biochemical abnormalities of HPA axis without the typical signs and/or symptoms of overt cortisol hypersecretion (11). Moreover, the development of SH has been described in up to 12% of AI patients after a mean follow-up period of 3 years (3,12-15). A spontaneous endocrine normalization of adrenal function has also been described in some AI patients with SH, while the progression to a clinical Cushing’s syndrome is rare (10, 14, 16, 17).

The diagnosis of SH is important since this condition has been associated with the presence of several cardiovascular risk factors, in particular obesity (OB), arterial hypertension (AH), type 2 diabetes mellitus (T2DM), dyslipidemia (DL) and bone damage (18-21).

Some studies (22-26), but not all (27, 28), reported an improvement of the metabolic complications of SH (AH, weight, and glycemic control) and of the quality of life after adrenalectomy, while a worsening of these conditions in untreated patients. As a consequence, the appropriate management of AI is actually a matter of debate (11, 29) and the utility of a long term follow-up of these patients has to be clarified (4, 30, 31). These uncertainties depend also on the lack of large longitudinal studies focused on “hard” end-points, such as major cardiovascular events (CVE) (11), even if it has been suggested that AI patients with SH are at risk of major CVE (32).

In this study we aimed to evaluate in a large population of AI patients with (SH+) and without SH (SH-) conservatively followed-up for at least 5 years the prevalence and the incidence of major CVE and the eventual changes of cortisol secretion and size of the adenoma over time.

Patients and Methods

Patients

We retrospectively analyzed the records regarding about 1400 AI patients referred to the Endocrine Units participating in the study between January 1996 and December 2012. Among these patients, about 300 have been operated on for the presence of overt hypercortisolism or for the size of the adenoma, 250 were not included due to the exclusion criteria and 300 to a follow-up <5 years, and 444 were lost at follow up. Eventually, 206 AI patients (144F, 62M) with a follow-up ≥5 yrs, without signs of overt hypercortisolism and in accordance with the exclusion criteria were enrolled. Sixty-five patients had been included in a previous cross-sectional study (33). We excluded patients with depression and alcoholism or other diseases, or treated with drugs influencing cortisol and dexamethasone metabolism or cortisol secretion, signs or symptoms of overt cortisol excess (i.e. moon facies, striae rubrae, skin atrophy or buffalo hump), history of malignant disease, infections, adrenal hemorrhage, pheochromocytoma, primary hyperaldosteronism, and infiltrative disease potentially affecting the adrenal glands.

All AI were discovered by radiological evaluations (abdominal and chest TC scan, abdominal ultrasonography or MRI) performed for unrelated diseases. Ultrasound findings were confirmed with unenhanced CT scan. All adrenal masses were ≥1 cm of diameter, and displayed a CT pattern consistent with a benign adenoma, such as a homogeneous texture with low density (<10 Hounsfield Units) and regular and smooth margins and size less than 6 cm (4, 11, 29). In patients with bilateral adenomas, the diameter of the largest adenoma was reported.

In all patients, we measured at baseline and at end of the follow-up: 24-hour urinary free cortisol (UFC) excretion, plasma ACTH levels at 08:00 h, and serum cortisol levels at 08:00 h after 1mg-dexamethasone suppression test (1mg-DST). We diagnosed SH in the presence of: 1mg-DST cortisol levels higher than 5 µg/dl (138 nmol/L) or in the presence of at least 2 of the following 3 parameters: ACTH<10 pg/mL (2.2 pmol/L), increased UFC and 1mg-DST cortisol levels >3.0 µg/dl (83 nmol/L). Increased UFC levels were defined by levels besides the upper limit of the normal values (ULN) of each assay. On the basis of these criteria, at baseline 24 patients (11.6%) had SH (SH+), while 182 had non secreting adenoma (SH-).

No SH+ patient at baseline showed a normalization of the adrenal function over time, while 15 SH- patients (8.2%), became SH at the end of the follow-up. Since we could not establish the exact time of occurrence of SH, these patients have been included in the SH+ group. Therefore, the SH+ and SH- groups were composed by 39 and 167 subjects, respectively.

Written informed consent was obtained by all subjects and the study was approved by the local Ethics Committees.

Methods

Given the retrospective design of the study, there was not a standardized protocol among the participating centers and we report data only at the beginning and at the end of the follow up. In all patients, the presence of obesity, hypertension, type 2 diabetes mellitus, dyslipidemia and the prevalence of major cardiovascular events (CVE) were evaluated at baseline. The changes in weight, glucose and lipid metabolism, blood pressure and the occurrence of incident CVE were evaluated at the end of the follow-up. We considered as major CVE the history or the occurrence of coronary heart disease or ischemic/hemorrhagic stroke. In the diabetic, dyslipidemic and hypertensive patients the prevalent and incident CVE, the blood pressure and the metabolic control were assessed by the reports of the cardiologists and diabetologists, who annually evaluated the patients. In the remaining patients, these information were directly obtained only at the beginning and at the end of the follow up by self-report. In the diabetic patients, the metabolic control was assessed by evaluating glycated haemoglobin.

AH was defined in the presence of systolic blood pressure of ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or antihypertensive treatment (34). T2DM was diagnosed using WHO criteria (35). Patients were considered affected with DL in the presence of serum triglycerides levels ≥150 mg/dL, or high-density lipoprotein (HDL) cholesterol levels <40 mg/dL in men and <50 mg/dL in women or if any specific treatment was given (36).

The improvement or worsening of body weight was defined by a greater than 5% change of body weight (37), between baseline and end of the follow-up period. The improvement or worsening of arterial blood pressure was defined if during the follow-up period the non-hypertensive patients passed from a

prehypertension category to another or the hypertensive patients from a hypertension grade to another, following the Guidelines for the Management of Arterial Hypertension of the European Society of Cardiology (34). AH was also considered improved or worsened if any antihypertensive treatment was interrupted or started respectively. Fasting glucose and LDL-cholesterol levels were considered improved or worsened if they passed from a category to another in agreement with the Adult Treatment Panel III criteria (36).

Serum and urinary samples were collected and stored at –20 C until assayed. In all patients ACTH and serum and urinary cortisol levels were measured in each Institute using commercially available reagents. The intra- and inter-assay coefficients of variation were <10% for all the assays.

Since the UFC normal values vary by using different assay, UFC values were expressed as the percentage difference between the detected value and the upper normal limit of each assay.

Statistical Analysis

Statistical analysis was performed by SPSS version 18.0 statistical package (SPSS Inc, Chicago, IL). The results are expressed as mean±SD, unless differently specified. Categorical variables were compared by $\chi 2$ test. Comparison of continuous variables among the different groups was performed using one-way ANOVA or Bonferroni test, as appropriate.

Bivariate associations between the diameter increase and the different indexes of cortisol secretion at baseline were tested by either Pearson product moment correlation or Spearman correlation, as appropriate.

The logistic regression analysis assessed the association between: i) the prevalence of CVE (dependent variable) and the presence of SH and the variables that were significantly different between SH+ and SH- patients at baseline; ii) the appearance of SH during the follow up (dependent variable) and the variables that were significantly different (or tended to be different, i.e. $P \leq 0.2$) between patients who remained SH- and patients who developed SH+ during follow-up; iii) the worsening of at least two metabolic complications, among the worsening of body weight, blood pressure, glycemic and LDL cholesterol control (dependent variable), and the presence of SH, age and duration of follow-up; iv) the incident CVE (dependent variable) and the presence of SH or of increased 1mg-DST cortisol levels, or UFC or ACTH<10

pg/mL (2.2 pmol/L) and the variables that were significantly different (or tended to be different, i.e. P≤0.1) between SH+ and SH- patients at follow-up.

The receiver operating characteristic (ROC) curve analysis was performed to assess the cut-off of the variables with the best diagnostic accuracy (sensitivity, SN; specificity, SP; positive and negative predicting value, PPV and NPV, respectively) for detecting SH- patients at risk for developing SH during the follow up. The same analysis was used to assess the best cut-off (s) of the 1mg-DST cortisol levels, and their SN and SP, for predicting the occurrence of incident CVE. P-values of less than 0.05 were considered significant.

Results

Clinical and biochemical parameters of SH+ and SH- patients, at baseline and at the end of follow-up are summarised in Table 1.

Baseline

BMI, gender and the presence of OB, AH and DL were comparable between SH+ and SH- patients. As expected, ACTH levels were lower, whereas UFC levels and 1mg-DST cortisol levels were higher in SH+ than in SH- group. In this latter group 11 patients showed 1mg-DST cortisol levels between 3.0 and 4.9 μg/dL (83-135 nmol/L), in the absence of other alterations of the HPA axis activity parameters.

The diameter of the adenoma was greater in SH+ than in SH- patients. The prevalence of bilateral adenoma was higher in SH+ compared to SH- patients. The side of the adenoma was 58.8% right and 41.2% left.

Overall, the 76.2% of patients were affected with T2DM and/or AH and/or DL. SH+ patients were significantly older and showed a higher prevalence of T2DM and CVE than SH-. Therefore we performed the logistic regression analysis including the presence of T2DM and age in the model. This analysis showed that SH and T2DM were independently associated with the presence of CVE (OR 3.1, 95%CI 1.1-9.0 and OR 2.0, 95%CI 1.2-3.3 respectively, $P<0.05$) regardless of age (OR 1.0, 95%CI 1.0-1.1, $P=0.553$). Finally, after excluding from the SH+ group the patients with a possible biochemically overt hypercortisolism (2 subjects with UFC levels >100% of ULN and 1 patient with ≥2 altered parameters among 1mg-DST cortisol levels >5 μg/dL, 50 nmol/L, ACTH <5 pg/mL, 1.1 pmol/L, and UFC >100% of ULN), the results did not change (data not shown).

Follow-up

Changes of cortisol secretion and size of the adenoma.

The mean duration of the follow up was 82.5±32 months (median, range: 72.3, 60-186 months), comparable between SH+ and SH- patients.

In SH+ patients, 1mg-DST cortisol levels significantly increased during the follow up, while ACTH and UFC levels were unchanged (Table 1). The diameter of the adenoma tended to increase over time. Five patients developed another adenoma at the contralateral adrenal gland (Table 1). A mass size increase of ≥1 cm was observed in the 8.3% of patients, whereas an increase >2.5 cm was observed in 2.4% patients. The parameters of adrenal function at baseline were not associated with the diameter increase (data not shown).

The Table 2 reports the comparison of the clinical and biochemical characteristics at study entry between SH- patients and the 15 patients who developed SH during the follow up. The same Table shows the comparison of the clinical and biochemical characteristics at end of follow up between the 15 patients who developed SH during the follow up and the 24 patients with SH diagnosed at baseline. At end of follow up, the patients with SH diagnosed at baseline and those with SH diagnosed during the follow up were similar for clinical and biochemical characteristics. Among the 15 patients who develop SH during the follow-up, 3 subjects experienced an incident CVE. At the baseline 2 out of these 3 patients showed 1mg-DST cortisol levels >1.8 μg/dL (50 nmol/L) and an adenoma size >2.5 cm, 1 patient ACTH levels <10 pg/mL (2.2 pmol/L), and no one high UFC values.

At the study entry, the patients who developed SH during the follow up showed a larger size of the adenoma (P=0.003) and tended to be more frequently affected with bilateral adenomas as compared with SH- patients (P=0.08). The cortisol secretion at baseline was comparable between SH- patients and subjects without SH at baseline but who developed SH during the follow up, even if 1mg-DST cortisol levels tended to be higher in the latter group (P=0.2). However, at diagnosis among the 15 patients who developed SH during the follow up, 9 (60.0%) showed 1mg-DST cortisol levels ≤1.8 μg/dl (50 nmol/L).

Since the SH- patients who became SH+ during the follow-up were different (or tended to be different) for the tumor size, the frequency of bilateral adenoma and 1mg-DST cortisol levels as compared with subjects who remained SH-, we performed a logistic regression analysis in order to assess the independent associations between these variables and the risk of SH occurrence. The analysis showed that the diameter of the adenoma was associated with the risk of developing SH (OR 2.97, 95%CI 1.37-6.44, P=0.006), regardless of the presence of a bilateral adenoma (OR 2.94, 95%CI 0.75-11.51, P=0.123), 1mg-DST cortisol levels (OR 1.16, 95%CI 0.61-2.23, P=0.653).

The ROC curve analysis showed that the cut-off of the adenoma size with the best diagnostic accuracy for detecting patients at risk for developing SH was 2.4 cm (SN 73.3%, SP 60.5%, PPV 14.3%, NPV 96.2%). Indeed, only 4 out of the 105 patients (3.8%) without SH at baseline and with an adenoma smaller than 2.4 cm developed SH during the follow up. On the other hand, 11 out the 77 patients (14.3%) without SH at baseline and with an adenoma ≥2.4 cm, in fact developed SH during the follow up. Among the 15 patients who developed SH during the follow up, a diameter increase > 1 cm was found only in one subject.

Cardiovascular and metabolic outcome

The occurrence of CVE and changes in body weight, blood pressure, glycemic and LDL cholesterol control in patients with and without SH at the end of follow-up are reported in Table 3.

In SH+ group, the prevalence of AH significantly increased during the follow-up. At the end of follow up, SH+ patients showed a higher prevalence of AH and T2DM than SH-, whereas the prevalence of dyslipidemia and obesity were comparable. The annual rate of CVE events (2.2% in the whole population) was higher in SH+ (3.1%) than in SH- patients (1.2%, P=0.004).

The weight and glycemic control and LDL cholesterol worsened in 26%, 25% and 13% of patients, respectively, without any difference between SH+ and SH- patients. The blood pressure control tended to worsen mainly in SH+ than in SH- group, even if the statistical significance was not reached (P=0.07). The worsening of at least 2 metabolic complications was higher in SH+ than in SH- group (53.8% vs 25.7%, P=0.001 respectively). The presence of SH was associated with the worsening of at least 2 metabolic complications (OR 3.32 95%CI 1.6-6.9, P=0.002), regardless of age (OR 1.03 95%CI 0.99-1.06, P=0.08) and the duration of follow-up (OR 1.01 95%CI 1.0-1.02, P=0.08).

At the end of follow-up, the SH+ patients experienced a higher number of CVE than the SH-. Seven (32%) of the 22 incident CVE occurred in patients with prevalent CVE at baseline and 15 (68%) in patients without prevalent CVE.

The logistic regression analysis showed that the presence of SH was significantly associated with the occurrence of incident CVE (OR 2.7 95%CI 1.0-7.1 P=0.04), but not with the worsening of blood pressure

control (OR 1.3 95%CI 0.5-3.2 P=0.634) and the duration of follow-up (OR 1.0 95%CI 0.99-1.01 P=0.842). The SN and SP of the SH diagnosis in predicting the incident CVE were 36.4% and 83.2%, respectively.

The 1mg-DST cortisol levels tended to be associated with the occurrence of incident CVE (OR 1.3, 95%CI 1.0-1.6, P=0.06), but not with the worsening of blood pressure control (OR 1.5, 95%CI 0.6-3.7, P=0.414) and the duration of follow-up (OR 1.0, 95%CI 1-1.1, P=0.708), while ACTH and UFC levels were not (data not shown). The ROC curve analysis confirmed this association (Figure 1) and showed that the cut-offs of 1mg-DST cortisol levels with the best compromise between SN and SP in predicting the occurrence of a new CVE were set at 1.5 μg/dL (41.4 nmol/L, SN 77.3%, SP 50%) and at 2.0 μg/dL (55.2 mmol/L, SN 50%, SP 68.5%).

Discussion

This is the first report of a large population of AI patients conservatively followed up for at least 5 years**.** After a follow-up period of at least 5 years, we observed that 8.2% of the patients with a normal adrenal function at baseline developed SH over time, with the risk being particularly increased in patients with an adenoma size ≥ 2.4 cm. The prevalence of CVE was significantly higher in SH+ than in SH- patients, and associated with SH regardless of age and presence of T2DM. The occurrence of incident CVE was significantly higher in SH+ than in SH- subjects, independent of the duration of the follow-up.

The present finding that 8.2% of AI patients develop SH over time confirms what has been reported in previous studies with a shorter follow-up. Indeed, depending on the different diagnostic criteria used to define SH, the development of this condition has been described in up to 12% of AI patients after a mean follow-up period of 3 years (3, 12-15,17). In accordance with some authors, in our series no patients showed a normalization of the adrenal function (7, 27), an issue that, however, is still debated (15, 16). Due to the design of the study and the fact that SH is clinically silent, we cannot precisely establish the time of the appearance of the biochemical alterations.

Previous findings suggested that tumors of 3 cm or greater are more likely to develop silent hyperfunction than the smaller ones (5, 38). In keeping, in the present study, the only variable significantly associated with the risk of developing SH was the diameter of the adenoma. Indeed, the 14.3% of patients with and adenoma ≥2.4 cm developed SH over time, regardless of the duration of the follow up, the presence of a bilateral adenoma and the degree of cortisol secretion at baseline. On the contrary, the risk of developing SH is low in patients with adenomas <2.4 cm.

However, although, at baseline, 1mg-DST cortisol levels were suppressed (≤1.8 μg/dL, 50 nmol/L) in about half of patients deemed to develop SH, 2 out of the 3 subjects who developed SH and showed an incident CVE during the follow up, had baseline 1mg-DST cortisol levels >1.8 μg/dL (50 nmol/L) and an adenoma size >2.5 cm. Overall, these findings suggests that, even in the absence of an established diagnosis of SH, the presence of an adenoma size ≥ 2.4 cm, particularly, if in the presence of 1 mg-DST cortisol levels

>1.8 μg/dL (50 nmol/L), may be an important element for deciding the biochemical and clinical follow up in AI patients.

In 8.3% of patients we observed an increase of the adenoma size of at least 1cm; only in 2.4% of cases the diameter increase was >2.5 cm. As expected, none of these cases was a malignancy (3). This prevalence confirms previous data obtained in smaller series, showing a >1cm increase of the adenoma size in 5-20% of patients (7, 8).

The novelty of the present study is the evaluation of the prevalence and incidence of "hard" end-points such as the major CVE after a long-term follow up. In accordance with a previous cross-sectional study (32), we found that SH patients are at increased risk of coronary heart disease and stroke. However, for the first time, we demonstrate that AI patients with SH are at risk of incident CVE. Interestingly, SH was an independent factor accounting for CVE though both hypertension and hyperglycemia became more prevalent with follow-up, with a rate of increase higher than in SH- patients. Even if the worsening of AH could have been a risk factor for incident CVE, the logistic regression analysis suggested that the worsening of blood pressure levels was not associated with the incident CVE when adjusting for the duration of the follow up and for the 1mg-DST cortisol levels or the presence of SH. However, since SH itself may lead to the worsening of AH, it is very difficult to discriminate the possible independent roles of the cortisol hypersecretion and the blood pressure controls in determining the risk of CVE in these patients.

As the appropriate management of AI is actually a matter of debate, our findings may contribute to define the utility of a long-term follow-up of these patients and to suggest an adequate management (4, 11, 29). Our data confirm that a long-term radiological follow-up should not be routinely undertaken, a recommendation that was largely based on meta-analysis of previous smaller series (6).

On the other hand, an clinical follow-up, carefully evaluating the cardiovascular risk should be done in these patients. The annual rate of CVE in SH+ patients (3.1%) in the present study, similar to that reported in the populations at risk, such as in diabetic patients (39), is not surprising, considering the high prevalence of patients with T2DM and/or AH and/or DL (76.2%) in the population enrolled in the present study.

The present study suggests that the occurrence of CVE, or worsening of cardiovascular risk profile may prompt an endocrine re-evaluation (40) and that patients with SH should be carefully followed-up. At this regard, the use of 1mg-DST cortisol levels as single parameter to predict the risk of CVE seems to have an acceptable sensitivity (77%) if a low cut-off is chosen (i.e. 1.5 μg/dL, 41.4 nmol/L) but at the expense of a low specificity (50%). The use of a combination of different HPA axis parameters, such as the one we choose in the present study, allows to increase the specificity (83.2%). Unfortunately, data regarding midnight serum cortisol levels were not available, since it requires the hospital admission (11), which is not widely available in Italy. In the absence of high quality prospective trials on the value of adrenalectomy, it has been proposed to consider surgery in younger patients with SH showing diseases potentially attributable to cortisol hypersecretion (AH, T2DM, OB, and osteoporosis) that are of recent onset, or are resistant to adequate medical treatment, or that are rapidly worsening (4, 30). The present findings demonstrate that the deterioration of metabolic and cardiovascular status may be fueled by SH. It is possible that in these patients surgery could avoid the occurrence of major cardiovascular diseases.

We disclose the limitation of the small number of SH patients, that severely affects the power for low-frequency events and for teasing out the influence of cortisol. In addition, the retrospective design of the study and the lack of a standardized protocol among the participating centers did not consent to obtain potentially interesting data (i.e. waist circumference) and might have led to a possible selection bias towards patients at overall better prognosis. It is likely that patients who were perceived to be at higher risk for CVE or presented with larger masses underwent adrenalectomy. However, this bias may have paradoxically reinforced the importance of our findings. Indeed, despite the fact that the patients included in the study may had been at low risk of CVE, we found that the cardiovascular risk was anyhow increased. Another unavoidable limitation of this study is related to the diagnosis of SH itself (11). Indeed, since the diagnosis of SH is defined by arbitrary criteria, it is possible that some patients classified as not having SH might have, in fact, a mild degree of cortisol hypersecretion. This may explain why CVE occur also in patients without SH and is in keeping with the finding that 11 patients classified as SH-, in fact, had 1 mg-DST cortisol levels between 3 and 4.9 μg/dL (83-135 nmol/L), in the absence of other alterations of the HPA axis activity parameters. In addition, it is well known that ACTH and cortisol assays are largely inaccurate at the low ends

(11, 41). At this regard, the lack of centralization of the hormone measurements is another limitation of the study, although we used a uniform definition of SH.

In conclusion, the present study provides two major findings that may influence clinical practice. Firstly, in AI patients without SH a clinical and biochemical long-term FU is recommended for the risk of SH development, especially in patients with an adenoma larger than 2.4 cm. Secondly, in AI patients with SH the increased risk of worsening of the metabolic control and, importantly, of CVE has to be taken into account in addressing the treatment of choice.

A case-finding approach to select patients for repeating endocrine work-up over time may identify patients who have a degree of cortisol excess that is clinically relevant and may benefit from adrenalectomy, while saving resources compared to a wide-scale endocrine follow-up.

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42.
43. **Table 1. Clinical and biochemical parameters of patients with and without SH, at baseline and at end of follow-up.**

	Baseline		**Follow-up**	
	SH- Group (n=167)	**SH+ Group (n=39)^**	**SH- Group (n=167)**	**SH+ Group (n=39)^**
Age (yrs)	58.5±10.1 (25-79)	62.2±11* (25-78)	65.3±9.9 (35-86)	68.5±11.0 (35-91)

Female	119 (71.3)	25 (64.1)	-	-
BMI (kg/m^2)	27.9±5.0 (17.3-44.7)	28.3±5.6 (19.4-47.0)	28.2±5.4 (17.0-52.1)	29.2±6.0 (19.3-49.6)
Obese subjects	46 (27.5)	13 (33.3)	54 (32.3)	18 (46.2)
Bilateral adenomas	18 (10.8)	11* (28.2)	22 (13.2)	12$^{\#}$ (30.8)
Diameter of adenoma (cm)	2.2±0.7 (1.0-4.0)	2.8±0.9* (1.5-6.0)	2.5±0.9 (1.0-6.0)	3.1±1.0*$^{\#}$ (1.6-6.0)
ACTH (pg/mL)	16.1±11.5 (2.0-78.0)	11.3±6.0* (3.0-28.0)	16.9±10.6 (1.4-72.0)	8.6±3.8*$^{\#}$ (3.0-19.8)
1mg-DST (μg/dL)	1.6±0.8 (0.2-4.9)	3.5±2.1* (1.1-9.3)	1.6±0.8 (0.1-4.1)	4.9±2.2*$^{\#\infty}$ (1.5-10.4)
UFC%	-42.0±36.0 (-90.9-134.0)	-14±68.5* (-85.7-266.0)	-39.6±34.4 (-93.3-111.4)	-14.1±50.7*$^{\#}$ (-89.1-121.3)
Hypertensive patients	90 (53.9)	26 (66.7)	105 (62.9)	34$^{\#*\infty}$ (87.2)
Diabetic patients	28 (16.8)	13* (33.3)	37 (22.2)	17*$^{\#}$ (43.6)
Dyslipidemic patients	70 (41.9)	21 (53.8)	90* (53.9)	27* (69.2)
Patients with prevalent cardiovascular events	10 (6.0)	8* (20.5)	-	-

44.

45. Data are mean ±SD with range in parenthesis or absolute number with percentage in parenthesis.

46. ^This group includes 15 SH- patients who became SH+ at the end of the follow-up. Since we could not establish the exact time of occurrence of SH, these patients have been included in the SH+ group also for baseline evaluations.

47. BMI: body mass index; 1mg-DST: serum cortisol levels after 1–mg dexamethasone suppression test; UFC%: percentage difference between urinary free cortisol (UFC) levels and upper limit of normal values (ULN). ACTH: adrenocorticotroph hormone. SH is diagnosed in presence of cortisol after 1mg-DST >5 µg/dl (138 nmol/L) or of 2 out of the following 3 parameters: ACTH <10 pg/mL (2.2 pmol/L); 1-mgDST >3 µg/dl (82.7 nmol/L); high UFC levels. SH+: patients affected with subclinical hypercortisolism (SH). SH-: patients without subclinical hypercortisolism. *P<0.05 vs SH- at baseline; # P<0. 05 vs SH- at follow-up; ∞ P<0.05 vs SH+ at baseline. SI conversion factors: cortisol x 27.59; ACTH x 0.22.

48. **Table 2. Comparison of the clinical and biochemical characteristics at baseline between the patients without subclinical hypercortisolism (SH) and the 15 patients who developed SH during the follow up, and at the end of follow up between the 15 patients who developed SH during the follow up and the 24 patients with SH diagnosed at baseline.**

	Baseline		
	Patients without SH (n=167)	**Patients who developed SH during the follow up (n=15)**	**Patients with SH d at baseline (n=**
Age (yrs)	58.5±10.1 (25-79)	59.3±12.6 (40-76)	70.4±10.5 (44-91)
Females	119 (71.3)	13 (86.7)	12 (50)
Duration of follow-up (months)	-	-	79.5±28.4 (60-178)
BMI (kg/m^2)	27.9±5.0 (17.3-44.7)	28.0±4.7 (20.2-36.3)	29.3±6.5 (19.3-49.6
Obese subjects	46 (27.5)	6 (40)	10 (41.7)
Bilateral adenomas	18 (10.8)	4 (26.7)	7 (29.2)
Diameter of adenoma (cm)	2.2±0.7 (1.0-4.0)	2.8±0.7* (1.5-4.0)	3.1±1.1 (1.6-6.0)
ACTH (pg/mL)	16.1±11.5 (2.0-78.0)	11.0±3.5 (4.0-15.0)	9.3±3.7 (3.8-19.8)

1mg-DST (μg/dL)	1.6±0.8 (0.2-4.9)	1.9±0.6 (1.1-3.2)	4.8±2.1 (1.5-10.4)
UFC%	-42.0±36.0 (-90.9-134.0)	-40.4±30.6 (-85.7-17.6)	-19.9±49.6 (-89.1-79.1
Hypertensive patients	90 (53.9)	10 (66.7)	21 (87.5)
Diabetic patients	28 (16.8)	5 (33.3)	11 (45.8)
Dyslipidemic patients	70 (41.9)	8 (53.3)	17 (70.8)
Patients with prevalent cardiovascular events	10 (6.0)	2 (13.3)	6 (25.0)

49. Data are mean ±SD with range in parenthesis or absolute number with percentage in parenthesis. BMI: body mass index; 1mg-DST: serum cortisol levels after 1–mg dexamethasone suppression test; UFC%: the percentage difference between urinary free cortisol (UFC) levels and upper limit of normal values (ULN). ACTH: adrenocorticotroph hormone. SH is diagnosed in presence of cortisol after 1mg-DST >5 μg/dl (138 nmol/L) or of 2 out of the following 3 parameters: ACTH <10 pg/mL (2.2 pmol/L); 1-mgDST >3 μg/dl (82.7 nmol/L); high UFC levels. SH+: patients affected with subclinical hypercortisolism (SH). SH-: patients without subclinical hypercortisolism. SI conversion factors: cortisol x 27.59; ACTH x 0.22. * P<0.005 vs SH- Group.

50. **Table 3. Occurrence of cardiovascular events and changes in body weight, blood pressure, glycemic and LDL cholesterol control in patients with and without subclinical hypercortisolism at the end of follow-up.**

51.

	SH- Group (n=167)	**SH+ Group (n=39)**	**P**
Duration of follow-up (months)	83.2±33.6	79.4±25.2	0.826

	(60-186)	(60-178)	
New CVE	14 (8.4)	8 (20.5)	0.040
New CVE in CVE- patients at baseline	11 (6.6)	4 (10.0)	0.343
Increased body weight (%)[1]	40 (24.0)	13 (33.3)	0.229
Worsened blood pressure control (%)[2]	52 (31.1)	18 (46.2)	0.070
Worsened glycemic control (%)[3]	39 (23.4)	12 (30.8)	0.334
Worsened LDL[3]	20 (12.0)	7 (17.9)	0.303

52.

53. Data are mean ±SD with range in parenthesis or absolute number of patient with percentage in parenthesis.

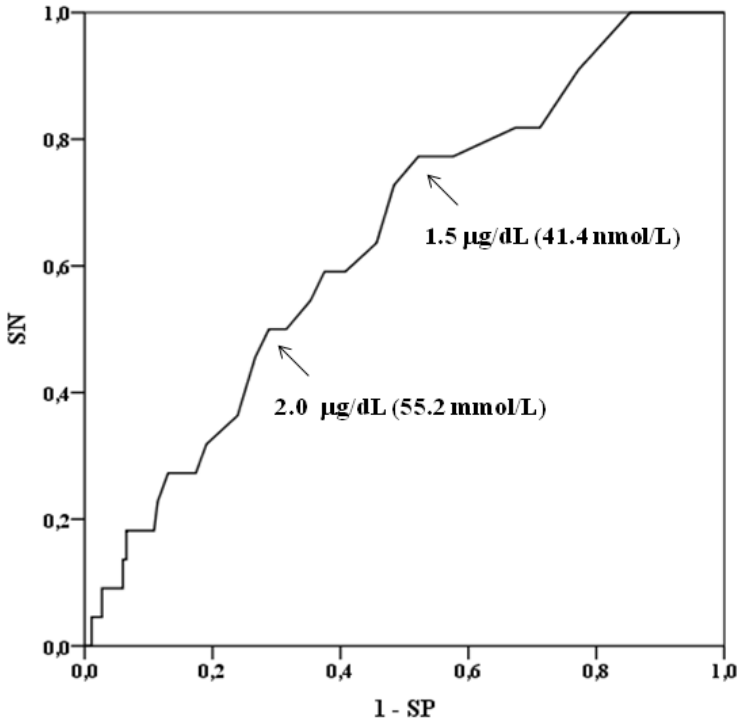
54. LDL: low density lipoprotein. CVE: cardiovascular events. CVE- patients: patients without previous CVE.

55. [1]Body weight is considered improved or worsened in the presence of at least a 5% variation in respect to baseline (36).

56. [2]Blood pressure levels were considered improved or worsened if it passed from a category to the other in agreement with the Guidelines of the European Societies of Hypertension and Cardiology (33).

57. [3]Fasting glucose and LDL-cholesterol levels were considered improved or worsened if they passed from a category to the other in agreement with the ATP III criteria (35).

Figure 1.



58. Legend to figure 1.

59. **Figure 1**. Receiver Operating Characteristic curve for cortisol levels after 1 mg dexamethasone suppression test (1 mg-DST) in predicting the occurrence of incident cardiovascular events (CVE). The cut-offs of 1mg-DST cortisol levels with the best compromise between sensitivity (SN) and specificity (SP) in predicting the occurrence of a new CVE are set at 1.5 µg/dL (41.4 nmol/L, SN 77.3%, SP 50%) and at 2.0 µg/dL (55.2 mmol/L, SN 50%, SP 68.5%), as indicated by the arrows.